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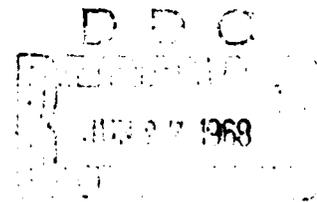
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MEDICOLEGAL INTEREST OF THE IMMUNOFLUORESCENT PREGNANCY TEST

Minerva Medicolegale
(Medicolegal Minerva)
Vol. 85, 1965, pp 61-64.

V. Querci, Assistant and University Lecturer, Institute of Forensic Medicine and Insurance, University of Siena (Director: Prof. Dr. M. Barni)

By now it is more or less proven that none of the biological pregnancy tests proposed up to now are capable of removing all diagnostic doubts in the field of clinical and medicolegal practice, and that not exceptionally they harbor within themselves results which are statistically predictable in 2% of the cases (Landgrebe and Hobson; Simas-Alves; Bevilacqua and formentano).

This negative behavior is due to two reasons: a) the receptor animals react in an analogous manner both to pituitary and to chorionic gonadotropin, and therefore positive results may also be obtained in certain gynecological pathological states (primary and secondary amenorrhea, castration, menopause, ovarian aplasia, etc.); b) -- and this is no less important, especially in medicolegal practice -- the reaction is positive only in the presence of a large quantity of gonadotropin (ca. 5,000 I.U. per liter) (Cuboni, Carèlla, Fucci and Chini).

In order to overcome these disadvantages there have been developed chemical methods of hormone determination, and also, with greater success, immunological tests (Wide and Gemzell; MacKean) based on the antigenic property of chorionic gonadotropin, demonstrated by Collip and Anderson, and by Zondek and Sulman.

Subsequently Lunefeld, Iserky and Shelesnyak, and in Italy Pasetto, Montanino and Donini, and Lo Stucchi have, by suitable

modification of the original method, made possible the routine application (agglutination of erythrocytes, on which gonadotropins have been adsorbed, on contact with antigonadotropin) of the immunological pregnancy test (IPT), promising of highly satisfactory results demonstrating the absolute validity of a method which, combining the highest sensitivity* with an absolute specificity, reduces the possibilities of error to negligible proportions. The parallel validity of the method for medicolegal purposes (diagnosis of prior abortion) was finally demonstrated by Marziano and by Carella, Fucci and Chini.

These indisputable advantages notwithstanding, the practical application of the IPT still entails some difficulties and uncertainties of interpretation, which are peculiarly linked to the indeterminate and in some cases controversial nature of the objective results (agglutination or sedimentation of the red cells); these, however, may be overcome, as has recently been demonstrated by Querci and D'Antona, by means of a special modification of the method by which the antigen-antibody reaction is made visible through the phenomenon of induced fluorescence. The direct individuation and microscope visualization (in Wood's light) of the antigen (gonadotropin adsorbed on red cells) have made it possible to verify the reaction between the antigen and the specific antibody labeled with a fluorochrome (in this case chorionic antigonadotropin conjugated with fluorescein isocyanate). In this particular application, the acquisition of fluorescence by the erythrocytes on which gonadotropin is adsorbed indicates the absence of gonadotropin in the urine under investigation; absence of fluorescence, on the other hand, indicates the presence of gonadotropin and the occurrence of an antigen-antibody reaction on the level of the biological fluid. The good results obtained up to now authorize me to characterize the new method (IFPT) as an objectively valid test, and to describe our subsequent investigations undertaken in order to determine the sensitivity of the test and its usefulness for well-known medicolegal purposes.

Materials and Method

The studies were carried out on the urine of 15 women who had had verified abortions during the first three months of pregnancy; they had a clearly positive Galli-Mainini test on admission to the clinic**, and the test remained positive during the

*It is possible to detect the presence of gonadotropin even when their titer in the urine is less than 1,000 I.U./liter (Carella et al.).

**I thank Prof. P. Spoto, Director of the Obstetrics and Gynecological Clinic of the University of Siena for having authorized me to undertake these investigations, and for his prodigious advice and suggestions.

2-3 days following the instrumental revision. From these patients a few cc of blood were taken every day in order to carry out similar tests on the serum. On each urine and serum sample there were carried out, from the day of admission to the day on which all tests were completely negative, a normal IPT and the same test with immunofluorescent correction (IFPT). Whereas for the first test commercially available reagents were used, for the second test I have made use of the same reagents but modified in a suitable manner, consisting, in short, of

- buffered isotonic solution;
- erythrocytes on which chorionic gonadotropin has been adsorbed (vials No 2 of IPT Serotest);
- antiserum (vial No 3 of IPT Serotest) conjugated with fluorescein isocyanate*, lyophilized;
- acetone extract of rat liver homogenate, obtained according to the method of Coons, Leduc and Connolly.

The procedure employed was as follows:

- The lyophilizate (antiserum conjugated with fluorescein isocyanate) was suitable reconstituted with buffered isotonic solution, and absorbed for 10 minutes by the acetone extract of rat-liver homogenate, using 50 mg of acetone extract per cc. After prolonged mixing, the menstruum was extracted by centrifuging, repeated three times. It is preferable to use small quantities of extract and follow the method of non-prolonged but repeated absorption, in order to permit the serum to come into adequate contact with the absorbing extract and thus bring about the removal of free fluorescein and the blockade of cationic and anionic groups capable of reacting with the tissue proteins, thereby eliminating the aspecific fluorescence.

- 0.05 cc samples of buffered isotonic solution were introduced into vial No 2 in order to suspend the lyophilized erythrocytes.

- To 0.05 cc of urine or serum under investigation was added 0.45 cc of the suitably prepared antiserum, and after agitation, the mixture was allowed to stand for 30 minutes at room temperature.

- After this period 0.40 cc of the liquid was introduced into vial No 2. The mixture was agitated and then allowed to stand at room temperature for 1 1/2-2 hours; then the erythrocytes on the bottom were determined as in the case of the

*The method used was that proposed by Coons et al., followed by the ISVT Sclavo.

immunobiological method, in this way acquiring an additional adequate control for the reaction.

- The red cells were subsequently subjected to repeated washing with buffered isotonic solution to remove the excess fluorescent serum. After the last washing the supernatant was discarded and a drop of the material was withdrawn by means of a fine pipette, deposited on a fluorescence-microscopic slide and covered with a thin cover slip.

The slide was observed in a Zeiss microscope illuminated with an Osram HBO 200 lamp (24,000 stilbs) using a BG 12 excitation filter and a No 53 absorption filter.

The test is positive when, in the absence of any fluorescence, the occurrence of conjugation between the antigonadotropin combined with the fluorescein and the chorionic gonadotropin present in the urine is demonstrated, confirming the existence of pregnancy.

The test is negative when an obvious fluorescence is displayed, conferred on the red cells by the antiserum not used up during the contact with the urine, which is thus free of gonadotropin (cf. micropathographic illustration in Minerva Medico-legale, 1964, p 17).

Results and Inferences

The results have, in effect, been satisfactory and in full agreement with the theoretical premises.

In fact the IFPT remained positive in 100% of the cases until the tenth day following the instrumental revision, whereas the IPT was positive in 100% of the cases only up to the sixth day (see Table 1 and Fig. 1).

From the 11th day on, the test started to become negative and became negative in all cases on the 18th day, whereas with the IPT the tests were all negative by the 15th day. It should be kept in mind that the usual biological pregnancy tests become negative in 100% of the cases already after three days.

At the same time the tests carried out with sera, using either the IPT or the IFPT, remained positive as long as the respective urine test was positive, and in many cases remained positive for an additional 24-48 hours.

Table 1.

Casi (2)	Garni di purpura post-abortivo																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15)	GM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ITC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	IFTG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

1 -- Days of post-abortive confinement; 2 --- Case; 3 -- Method; GM = Galli-Mainini; ITG = IPT; IFTG = IFPT.

Thus the case material examined -- even if quantitatively very limited -- sufficiently demonstrates the validity of the IFPT not only from the merely technical point of view of greater certainty and objectivity of the results, but also on the diagnostic level, in view of the fact that not infrequently its sensitivity is much higher than that of the usual biological tests and even of the immunological tests (IPT) which, as has been shown by Carella Fucci and Chini, stops being positive below a concentration of 1,000 I.U. Obviously the IFPT is capable of giving unequivocally positive reactions even at a much lower gonadotropin level; thus, it seems legitimate to call attention to its greater diagnostic sensitivity and utility even after the IPT has become negative. This undoubted advantage is obviously due to the more sensitive system of reading off the results, based on the control and interpretation of a distinctly visible phenomenon -- the fluorescence of the test erythrocytes -- which is of course preferable to the phenomenon of agglutination of the erythrocytes on which the IPT is based, which is possible even after a partial neutralization of the antibodies had taken place*.

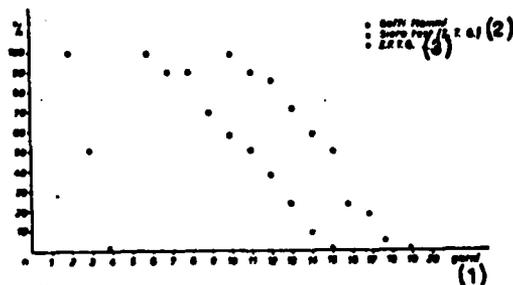


Fig. 1.

1 -- Days; 2 -- Serum test (IPT); 3 -- IFPT.

It is true that an incomplete utilization of the antibody on the part of the gonadotropin present in the test samples may give rise to an uncertain and reduced fluorescence of the erythrocytes; this, however, is readily distinguishable from a

*In fact, where the gonadotropin concentration in the urine or serum is very low, there is very little neutralization of the antigonadotropin, whence the residual antigonadotropin is still capable of inducing the agglutination of the erythrocytes, thus suggesting the absence of pregnancy even when a state of pregnancy does in fact exist, and the low gonadotropinuria and gonadotropinemia do not permit a complete neutralization of the antigonadotropin.

..
distinct fluorescence which, alone, makes it justified to rule out the possibility of existence of pregnancy.

To conclude, it is now clearly established that the IFPT, despite a certain technical complexity which impedes its routine application, possesses the characteristics of an absolutely certain diagnostic test and is recommendable in those conditions of obstetrical and medicolegal interest which cannot be solved unequivocally by means of the usual tests.

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